Antifungal Finishing of Fabrics with Natural Dyes from Aerial Biomass of *Perilla frutescens* (L.) Britton

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**ABSTRACT**

Increased environmental consciousness coupled with detrimental impacts of synthetic dyes and consumers' concern over hygiene, cleanliness and protection, the demand for functional textiles has increased in recent years. Antimicrobial finishing imparts protective properties to textiles besides attractive shades. The study was aimed to determine the antifungal activity of colourants from aerial biomass of *Perilla frutescens* and dyed fabrics against pathogenic fungi infesting textiles materials. Antifungal activity of natural dye extracted from aerial biomass of *Perilla frutescens* against the selected pathogenic fungi viz., *Aspergillus niger*, *Aspergillus flavus*, *Fusarium moniliforme*, *Fusarium solani*, and *Penicillium decumbens* was evaluated by agar-well diffusion method. The MIC of the natural dye against each of the test fungi was determined by broth dilution method. Dyed silk, wool and cotton fabrics were also evaluated for antifungal activity by standard method. The natural dye showed antifungal activity against all the five test fungi in a concentration dependent manner. The treatment dose of 1000µg/ml recorded the highest growth reduction in all the test fungi, nearly at par with the positive control. The MICs of natural dye against the test fungi ranged from 32.39 to 36.50µg/ml. All kinds of dyed fabrics also showed remarkable antifungal efficacy against the test fungi. Dyed wool fabric exhibited the maximum growth reduction followed by silk and cotton. The result of the study demonstrated the remarkable antifungal activity of natural dye from *Perilla frutescens* aerial biomass and dyed fabrics. Therefore, *Perilla frutescens* can be considered as a potential source of natural dye with functional properties and can be used in protective finishing of different kinds of textile fabrics.

**Keywords:** Antifungal activity, Dyed fabrics, Functional textiles, Natural dye, *Perilla frutescens*.

1. **INTRODUCTION**

Natural dyes derived from plants, insects, animals and minerals have been used for textile colouration since antiquity. In recent years, increased environmental consciousness among consumers coupled with detrimental impacts of synthetic dyes on environment and human health, interest towards natural dyes has gained momentum. Furthermore, owing to environmental changes, consumers are becoming more concerned about hygiene, cleanliness and protection thereby increasing demand for functional clothing. Textile materials offer a favourable environment for growth and spread of microorganism owing to their large surface area and moisture retention ability. Microbial activity on textiles not only exert detrimental impact to textiles itself leading to unpleasant odour, discoloration, waning of the substrate, but also cause dermal infection, allergic reaction and associated diseases to the wearer. This has posed a major challenge for the researchers to address the issue through protective textile finishing. Natural dyes in addition to their colouring properties exhibit antimicrobial properties contributing to the longer life of the products on which they are applied. Antimicrobial finishing of textiles prove their functionality which may have diverse applications in the field of health, hygiene and medical textiles, aside from healthy vesture.

A number of plants have been investigated as a source of natural dye having ability to impart functional properties to textiles such as antimicrobial, insect repellent,
deodorizing, and UV protective effect, besides imparting attractive shades. On account of the presence of potent bioactive phytochemicals, natural colourants hold immense promise for developing antimicrobial textiles for aesthetic, hygienic, and medical applications. Therefore, natural dyes with functional properties have emerged as a high priority area in textile finishing and a key factor for protective clothing.

*Perilla frutescens* (L.) Britt. commonly known as Bhanjira is an annual ornamental plant in the Lamiaceae family. It is native to Asia growing along road sides, ditches, forest margins, and on hillsides. It is commonly grown as a low hedge-plant and is found throughout the hotter parts of India, Burma & Malaya, and extends westwards to tropical and south Africa. *Perilla* is a traditional crop of China, India, Japan, Korea, Thailand, and other Asian countries. Different parts of the plant have been used as an important traditional herbal medicine for treating various disease including depression, anxiety, tumor, cough, asthma, allergy, intoxication and some intestinal disorders.

*P. frutescens* is extensively investigated for its chemical constituents and a number of different phytochemical compounds including phenolic acids (rosmarinic acid, rosmarinic acid-3-O-glucoside, caffeic acid, caffeic acid-3-O-glucoside, ferulic acid); flavonoids (catechin, apigenin, apigenin-7-O-glucuronide, apigenin-7-O-diglucuronide, luteolin, luteolin-7-O-glucuronide, luteolin-7-O-diglucuronide, scutellarein, scutellarein-7-O-glucuronide, scutellarein-7-O-diglucuronide), anthocyanins (shisonin, malonylshisonin, cyanidin 3-O-cafeoylglucoside-5-O-glucoside, cyanidin-3-O-cafeoylglucoside-5-O-malonyl glucoside), lipophilic and volatile compounds, triterpenes, phytosterols, fatty acids, tocopherols, and policosanols have been reported from different parts of the plant. Leaves are rich source of carotenoids.

The plant has been pharmacologically investigated for its chemical activities, emollient, expectorant, stomachic, carminative, antiasthmatic, antitussive, antipyretic, antiasthmatic, antidote, antioxidant, antispasmodic, diaphoretic, emollient, expectorant, stomachic, carminative, then thoroughly cleaned under running tap water and finally dried under shade. Air dried plant materials were ground to fine powder (50 mesh) with an electric grinder and stored in sterilized cellophane bags in a cool dry place till further use.

### 2.3. Processing of plant material

The plant material was at first surface sterilized with 0.1% mercuric chloride (HgCl₂) immediate after collection, then thoroughly cleaned under running tap water to remove dirt and finally dried under shade. Air dried plant materials were ground to fine powder (50 mesh) with an electric grinder and stored in sterilized cellophane bags in a cool dry place till further use.

### 2.4. Textile substrates

Different kinds of fabrics including silk, wool and cotton were purchased from Gandhi Ashram, an authorized outlet of Khadi and Village Industries Commission (KVIC), Dehradun, India. All fabrics were washed with non-ionic detergent (1% on weight of fabric) for 30 min to remove starch and other impurities, then rinsed and dried at room temperature. The scoured fabrics were drenched in water for 30 min before dyeing.

### 2.5. Extraction of natural dye

Extraction of natural dyes from the aerial biomass of *Perilla frutescens* (PFAB) was at first carried out in acidic, alkaline and neutral mediums and optical density of dye extracts were measured. Based on the highest value of optical density, the alkaline medium was found to be most advantageous medium for extraction of natural dye. The parameters like material to liquor (water) ratio (MLR, g/100 ml), alkali content (%), and extraction time (min) for natural dye extraction from the PFAB were optimized by varying one parameter at a time and keeping the other parameter(s) constant followed by optical density measurement. Based on the highest value of optical density for a particular set of experiment, the optimum values of MLR, alkali content, and extraction time were determined as 15.5 g/100ml, 0.3%, and 45 min respectively for extrac-
tion of dye from PFAB. Natural dye from PFAB was extracted under optimized conditions. Briefly, powdered plant material was taken in a beaker and alkaline distilled water (0.3% Na₂CO₃) was added to it. The extraction was done for 45 min at the boiling temperature. The extract obtained was allowed to cool at room temperature and then filtered with Whatman (No. 1) filter paper. The filtrate was distilled under reduced pressure and finally dried over dehydrating agent in vacuum that resulted in natural dye powder (15.8%).

2.6. Phytochemical screening of natural dye

The natural dye obtained from PFAB was extracted successively with ethylacetate, acetone, methanol and distilled water and then respective extracts were subjected to qualitative phytochemical screening to detect the presence of different phytochemicals, by following standard protocols. All the qualitative phytochemical tests were replicated thrice for confirmation.

2.7. Dyeing of fabrics

Dyeing process variables including dye concentration, pH and dyeing time for different fabrics was optimized through experimental trials and the optimum values of dye concentration, pH and time were determined as 1.2%, 4.0 and 60 min for silk; 0.2%, 2.0 and 60 min for wool and 0.2%, 3.0 and 90 min for cotton respectively. Dyeing of different fabrics including silk, wool and cotton was performed under optimal condition of concentration, pH and time. After dyeing, the dyed fabrics were washed with 5g/l non-ionic detergent and then rinsed with water and air dried at room temperature.

2.8. Evaluation of antifungal activity

2.8.1. Preparation of test solutions

Test solutions of a series of concentrations viz., 50, 100, 200, 300, 400, 500 and 1000 µg/ml were prepared by dissolving PFAB natural dye in Dimethyl sulfoxide (DMSO). All test solutions were kept in refrigerator at 4°C till further use.

2.8.2. Fungal strains

Antifungal activity of the PFAB natural dye and dyed fabrics were determined against standard strains of pathogenic fungi namely Aspergillus niger (AN), A. flavus (AF), Fusarium moniliforme (FM), Fusarium solani (FS), and Penicillium decumbens (PD) considering their clinical and pharmacological significance. The test fungi were isolated from mould affected fabric materials during monsoon season and were identified on the basis of their growth characteristic, mycelial morphology, spore morphology and other important characters using standard protocol. All the fungal strains grown in pure culture were maintained in potato dextrose agar (PDA) culture slants at 4°C and were used as stock culture throughout the study.

2.8.3. Preparation of fungal inoculum

Spore suspensions were prepared in 0.9% saline water using cultured slant. The fungal spore suspension was adjusted to give a final concentration of 1x10⁵ cfu/mL.

2.8.4. Preparation of media

The medium was prepared by dissolving Potato dextrose agar (PDA) (HiMedia) in distilled water and autoclaving at 121°C for 15 minutes. For antifungal assay, 20 ml of sterile PDA media was poured in sterilized petridishes (9 cm diameter) with an equal thickness and allowed to solidify.

2.8.5. Antifungal assay of natural dye

Antifungal activity of the PFAB natural dye was determined by agar-well diffusion method. Spore suspensions (0.2 ml) were applied and uniformly spread on the surface of the pre-sterilized and autoclaved PDA petridishes using a sterile glass spreader. Wells of 6mm diameter were made in centre of each PDA petridishes with the help of sterilized cork borer. The wells were filled with test solutions of natural dye with three replications for each treatment. Control experiments were carried out under similar condition by using Griseofulvin as positive control and DMSO as negative control. All the petridishes including treatments and controls were allowed to diffuse for 2 hours and then incubated at room temperature (28±2°C) for 72 hours. After incubation, the antifungal activity of dye solutions was measured and expressed in terms of diameter (mm) of zone of inhibition.

2.8.6. Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined by broth dilution method. Fungi were first grown in the potato dextrose broth for 24 hrs and then inoculums were diluted five times (10⁻⁵ dilution) to control its vigorous growth. Then each test tube was added with 1.8 ml of potato dextrose broth and different concentrations (10⁻⁵ to 10⁻³ µg/ml) of natural dye separately followed by inoculation of 0.2 µl of respective fungi and kept at 28°C for 48 hrs. The tubes were examined for visual turbidity. The MIC was determined based on lowest concentrations of the extracts showing no turbidity (without microbial growth).

2.8.7. Antifungal assay of dyed fabrics

The antifungal activity of the dyed fabrics (silk, wool and cotton) against pathogenic fungi, AN, AF, FM, FS, and PD was quantitatively evaluated by reported method. Circular discs (5.00 ±0.1 cm dia.) of dyed fabrics were
placed in PDA plates and sterilized for 15 min at 121°C. Spore suspensions (1000 µl) of each fungus were added to the centre of fabric discs and incubated for 24 hr at 37±1°C. Test solutions of natural dye were made through ten-fold serial dilutions. A fixed volume of each dilution (100 µl) was inoculated on PDA plates and the plates were incubated at 37±1°C for 24 hrs. Untreated circular fabric discs of same dimension were taken as control. Radial diameter (mm) of fungal growth on the agar plates (control & treatment) was measured and the percentage of reduction in the fungal growth was calculated using following formula:

\[ R(\%) = \frac{A-B}{A} \times 100 \]

Where \( R \) = Reduction in fungal growth; \( A \) = Fungal growth on the control (untreated fabrics), and \( B \) = Fungal growth on the treated fabrics.

3. RESULTS

3.1. Phytochemical screening of natural dye

The ethylacetate, acetone, methanol and aqueous extracts of the PFAB dye were qualitatively screened to detect presence of different classes of phytochemicals. The results of qualitative phytochemical tests of the different extracts are presented in Table 1.

The qualitative phytochemical screening recorded the presence of various group of phytochemicals in the different extracts of natural dye. The results presented in Table 1 indicated the presence of terpenoids, steroids, flavonoids, phenolics in ethylacetate extract; terpenoids, steroids, flavonoids, phenolics and glycosides in acetone extract; steroids, terpenoids, flavonoids, phenolics, anthocyanins, tannin, glycosides, saponins, and amino acids in methanol extract and phenolics, flavonoids, anthocyanin, tannins, glycosides, saponins, carbohydrates, proteins, and amino acids in aqueous extract.

3.2. Evaluation of antifungal activity of natural dye

The antifungal efficacy of PFAB natural dye was evaluated in term of their zone of inhibition against altogether five pathogenic fungi and the results (zone of inhibition) were compared with the activity of a commercial fungicides, Griseofulvin taken as standard. The results of the assay are summarized in Table 2.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Extracts</th>
<th>Ethylacetate</th>
<th>Acetone</th>
<th>Methanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Phenolics</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthocyanin</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Proteins</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
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<tr>
<td>Amino Acids</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

(+): Present, (-): Absent

Table 2: Antifungal activity of PFAB natural dye against test fungi

<table>
<thead>
<tr>
<th>Natural Dye Conc (µg/ml)</th>
<th>Zone of Inhibition (in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AN (±)</td>
</tr>
<tr>
<td>50</td>
<td>3.85 ± 1.05</td>
</tr>
<tr>
<td>100</td>
<td>5.43 ± 0.57</td>
</tr>
<tr>
<td>200</td>
<td>11.67 ± 1.25</td>
</tr>
<tr>
<td>300</td>
<td>19.59 ± 1.09</td>
</tr>
<tr>
<td>400</td>
<td>25.69 ± 0.69</td>
</tr>
<tr>
<td>500</td>
<td>30.53 ± 1.31</td>
</tr>
<tr>
<td>1000</td>
<td>42.25 ± 0.67</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>44.59 ± 1.33</td>
</tr>
<tr>
<td><strong>DMSO</strong></td>
<td>−</td>
</tr>
</tbody>
</table>

It is evident from data presented in Table 2 that different test concentrations of PFAB natural dyes exhibit varying degree of antifungal activity against all the five fungal species in a concentration dependent manner. However, the test concentration of 1000 µg/ml of PFAB dye recorded the highest reduction in the growth of all the fungal species under study. Further, the antifungal activity of PFAB dye against all the five test fungi at the highest test concentration is found almost at par with the positive control. Minimum growth inhibition in all the test fungi is recorded with concentration of 50µg/ml. Increase in growth inhibition of all the test fungi was observed with increase in test concentrations of PFAB dye. Of different tested concentrations of the dye, inhibition of radial growth in all the test fungi was low at concentration of 50, 100 and 200 µg/ml, moderate at 300 and 400 µg/ml, and high at 500 and 1000 µg/ml (Fig. 1).

The mean radial growth inhibition of fungi AN, AF, FM, FS and PD with various concentrations of PFAB natural dye ranged between 3.85–42.25, 2.37–40.73, 3.16–41.29, 2.75–40.19, and 3.79–44.25mm respectively. Results indicated that all the treatments are very effective as compared to negative control. Treatment concentration of 1000 µg/ml showed the maximum radial growth inhibition whereas 50 µg/ml treatment concentration of natural dye recorded the minimum inhibition (Fig. 1).

3.3. Determination of MIC

The minimum inhibitory concentration (MIC) is the lowest concentration able to inhibit any visible fungal population. The MICs of the natural dye against test fungi AN, AF, FM, FS, and PD was recorded as 35.65, 34.89, 36.50, 32.39 and 34.27 µg/ml respectively. MIC is regarded as measurement of the activity of an antifungal agent against a fungus that confirms resistance of pathogenic fungi to an antifungal agent.

3.4. Evaluation of antifungal activity of dyed fabrics

Considering the excellent antifungal activity of PFAB dye, it was considered worthwhile to examine the different kinds of fabric dyed with PFAB dye for their antifungal property. Silk, wool and cotton fabrics dyed with PFAB dyes were evaluated for antifungal efficacy against the five test fungi and was measured as percentage reductions in fungal growth as shown in Table 3.

Results presented in Table 3 revealed that silk, wool and cotton fabrics dyed with PFAB dye have considerable antifungal efficacy against all the test fungi as reflected from reduction in fungal growth. Dyed wool fabric exhibited highest reduction in fungal growth in AF whereas maximum growth reduction was exhibited by dyed silk and cotton fabrics in AF and PD respectively. Dyed wool fabric showed highest growth reduction in all the five fungi under examination followed by dyed silk and cotton fabrics as observable from Fig. 2. Dyed cotton fabrics however showed the minimum growth reduction in all the test fungi.

4. DISCUSSION

The detected phytochemicals are reported to exhibit diverse biological functions, therefore hold high therapeutic importance. Presence of phenolics, flavonoids, anthocyanins and tannins in the dyes extract as observed in phytochemical screening is indicative of its excellent dyeing characteristics. Results of agar-well diffusion assay of PFAB dye against test fungi cultures (Fig. 1.), and the fungal growth reduction by different dyed fabrics (Fig. 2.) was an indication of promising antifungal potency of PFAB dye. In the recent years, considerable research work has been done across the world towards the application of natural dyes in coloration and antimicrobial finishing of textiles. In addition, antifungal activity of phytochemicals like phenolic compounds

![Fig. 1: Radial growth inhibition in test fungi by different doses of PFAB natural dye](image)

![Fig. 2: Growth reduction in test fungi by different fabrics dyed with PFAB dye](image)

| Table 3: Antifungal activity of dyed fabrics against test fungi |
|---|---|---|---|---|
| Dyed fabric substrates | Reduction in fungal growth (%) |
| | AN | AF | FM | FS | PD |
| Silk | 72.45 | 72.83 | 73.33 | 71.36 | 72.59 |
| Wool | 79.63 | 82.55 | 81.49 | 79.78 | 81.25 |
| Cotton | 67.39 | 65.76 | 65.49 | 63.69 | 66.23 |
including flavones and flavonoid glycosides, coumarins, and anthraquinones have been studied.39,40 Natural dyes of plant origin having tannins are reported to exhibit antibacterial and antifungal activity.41 The antifungal potency of natural dye derived from P. frutescens aerial biomass may be due to presence of phenolic compounds like phenolics, flavonoids, tannins, and anthocyanins of complex molecular structure and varied mechanisms of action. However, further studies are needed to investigate the PFAB natural dye for its chemical constituents responsible for its dyeing and antifungal characteristics.

5. CONCLUSION

Natural dyes of plant origin are reported to exhibit various medicinal and protective properties. The study mainly focuses on the antifungal activity of natural dye isolated from aerial biomass of P. frutescens as well as silk, wool and cotton fabrics dyed with PFAB dye. Results of the present study showed that the natural dye derived from aerial biomass of P. frutescens as well as fabrics dyed with it have demonstrated considerable antifungal activity. The antifungal assays demonstrate an exciting opportunity for, the textile materials dyed with PFAB dye that can be very useful in developing protective clothing to protect users against common infections. Thus P. frutescens apart from its traditional pharmacological properties can also be a potential source of natural dye with functional properties that could be beneficial for protective finishing of different kinds of textile fabrics.

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